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Foraging and mate-finding in the silver Y moth, *Autographa gamma* (Lepidoptera: Noctuidae) under the risk of predation

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Animal foraging and reproductive behaviour is influenced by other simultaneous demands such as predator avoidance. The trade-offs between these demands may depend on sex or mating experience. This study demonstrates that the olfactory-mediated foraging and mate-seeking behaviours in the silver Y moths, *Autographa gamma*, are affected by auditory cues mimicking their bat predators. Both males and females changed their foraging behaviour under simulated predation risk. Fewer moths reached the odour source following sound stimulation and the time to find the odour source increased by up to 250%. However, there were no significant differences between male and female ability to reach the plant odour source or the duration of the flight towards the source when stimulated with ultrasound. Hence females are not more cautious than males when observed in the same behavioural context. Risk-taking in males was independent of whether they were flying toward a flower odour or sex pheromones having equal attractive value. This indicates that the trade-off between olfactory and acoustic cues is independent the type of odour. Mated females were not as strongly affected by sound as non-mated, indicating that flower odours have a higher adaptive value for mated females, suggesting that some processes following mating experience influence the trade-off between flower odours and simulated bat sounds.


Conspicuous behaviour such as mate-finding or foraging exposes animals to predation. Thus, reproductive and foraging behaviour may conflict with predator defence behaviour. Several experiments have shown that risk of predation can alter decision-making in foraging animals (Sih 1982, Mittelbach 1984, Dukas 2002) and a few studies have described the trade-off between the need for energy intake and the risk of predation (Abrahams and Dill 1989, Magnhagen 1991). It has also been shown that conflicts can occur in animals confronted with simultaneous demands for predator avoidance and reproduction (Magnhagen 1990).

In general, studies on this topic have examined risk-taking to predators in relation to either mate-finding behaviour or foraging behaviour. However, the level of risk-taking might depend on the behavioural context in which the animal is involved and the sex or the mating status of the animal. For example, males that are engaged in sexual behaviour would be expected to take higher risk than when they are foraging. Some evidence suggests that risk-taking is sex-biased, since males are often more heavily preyed upon than females (reviewed by Lima and Dill 1990). Sex-biased predation has been reported since Darwin (1871) in moths, water striders, milkweed beetles, tungaro frogs and squirrels (Polis et al. 1998). Echolocating insectivorous bats in the wild catch male moths more frequently than females (Acharya 1995), which could be due to either increased risk-taking by male moths in accordance with predi-
tions from sexual selection theory or by increased activity level. Some female behaviour, like attraction to calling males or carrying of eggs, may lead to increased risk of predation (Lima and Dill 1990). Studies designed to separate risk-taking from general activity levels are thus necessary to address the question of sex-bias.

Typical nocturnal activities in moths include foraging and mate-finding. Adult moths in general rely only on nectar as a food source. The silver Y moth, Autographa gamma, is mostly active at dusk, but it can be seen visiting flowers at daytime and night as well (D. Plepys pers. obs.). Both males and females are sensitive to fragrances of flowers and actively search for those providing nectar. For mate location, males of *A. gamma* orient towards a pheromone-releasing female conspecific (Tóth et al. 1983, Mazor and Dunkelblum 1992). While engaged in these olfactory-induced activities moths must defend themselves against their most common predator, echolocating bats. Bats hunt their insect prey by use of sonar generally operating in the frequency range from 20–100 kHz. They scan the environment for potential prey by pulsed sound and listen for returning echoes. Most moths are equipped with a pair of ultrasound sensitive ears by which they can detect echolocating cries of insectivorous bats (Roeder 1967, Miller and Surlykke 2001). Evolution of bat detection in moths was combined with a set of behavioural manoeuvres aimed to avoid attacking bats. Roeder (1967) described a variety of behavioural responses of freely flying moths to artificial bat sound like turns away from the sound source, loops, rolls, spirals, dives, zigzag flight, etc. The neurophysiological mechanisms of ultrasound detection in moths are well described. However, the corresponding defence behaviours elicited by ultrasound arise from studies of moths in stationary flight (Fullard 1979, 1984, Skals and Surlykke 2000) or from field studies (Roeder 1967) where the behavioural context is unknown and where it is difficult to control the sound stimuli. Baker and Cardé (1978) showed that male pheromone flight could be disrupted by ultrasound stimulation from jingling keys. Acharya and McNeil (1998) found that pheromone-based mate searching behaviour in moths depends on the level of predation risk. Male moths exposed to simulated bat cry responded more frequently when the stimulus resembled a signal of high predation pressure than when the signal indicated low risk of predation. Likewise, female calling behaviour was affected depending on if the stimulus resembled an aerial hawking bat or a gleaning bat. However, Acharya and McNeil (1998) did not test if there was a difference between male and female response to the predatory signals. Female moths also take risks when exposing themselves to bat predation when foraging for nectar or searching for oviposition sites in the night and recently Svensson et al. (in press) demonstrated that mated females respond to ultrasound when flying toward oviposition sites. Therefore it is not straightforward to predict which sex of moths that takes the higher risk of predation.

It has been shown that the optimal trade-off between conflicting demands, such as foraging and predator defence, depends on the behavioural context (Rothley et al. 1997). We decided to test if risk taking is sex-biased in the silver Y moths when they are in the same behavioural context, i.e. by exposing both sexes to sounds mimicking an attacking bat while they were attracted toward a plant odour in a flight tunnel. Furthermore we tested whether risk taking in males depend on if the male is engaged in reproductive behaviour (mate location by pheromone-mediated flight) or foraging behaviour (flying towards flower odours). Finally we tested if mated females took higher risk than non-mated upon approaching a plant odour source.

**Materials and methods**

**Insects**

Males and females of the silver Y moth (*Autographa gamma*) were obtained from a laboratory culture initiated in 1997 and supplied with wild insects every summer. Larvae were reared on a semi-synthetic bean diet (Zhu et al. 1996) and kept at 23°C and 70 ± 10% RH on a reversed L17:D7 light cycle. Pupae were separated according to sex. Emerged males and females were kept in separate environmental chambers at the above-mentioned conditions. Adults were provided with water only. Experiments were carried out with 2–3 day old moths. Mated females for experiments were obtained by introducing one- or two-day old females to males in cages. During the first two hours in the dark phase they were observed for mating. Those that mated were tested in the flight tunnel on the next day after mating.

**Flight tunnel**

The experiments were carried out in a 3.0 × 0.90 × 0.90 m flight tunnel at white light illumination from above at 1–2 lux intensity. The air was pushed through the tunnel by a fan and the odour plume was ventilated out of the tunnel at the downwind end by an exhaust fan. The wind speed in the tunnel was kept constant at 0.3 m/s. The shape and position of the plume was visualised with TiCl₄, which forms smoke upon the reaction with air humidity. The experimental conditions were as follows: temperature 20–22°C; relative humidity 40–80%.
Preparation of odour sources

We used rubber septa (Thomas Scientific, Swedesboro, N.J., USA) as dispensers to release odours. Dispensers were prepared by adding 200 μl of hexane solution of either the pheromone blend or the floral odour phenylacetaldehyde (PAA) at concentrations appropriate for the experiments. The pheromone blend consisted of (Z)-7-dodecenyl acetate (Z7-12:OAc) and (Z)-7-dodecenol (Z7-12:OH) in a 1:0.05 ratio (Mazor and Dunkelblum 1992). Between experiments, dispensers were kept in closed vials at −20°C.

Dose-response experiment

We performed dose-response experiments with PAA and the pheromone blend. Both sexes were tested for their attraction to PAA and males only for their attraction to the above mentioned pheromone blend. To test attractivity of PAA we used the following doses: 0.001 mg, 0.01 mg, 0.1 mg, 1 mg and 10 mg. As control we used a blank rubber septum. Two doses of the pheromone blend, 0.25 μg and 2.5 μg respectively of Z7-12:OAc, where tested to see which one of them would match the attractiveness of one of the PAA concentrations ditto.

A rubber septum impregnated with the test odour was placed 43 cm above the tunnel floor at the upwind end of the flight tunnel. Moths were released individually into the plume from screen cages at the downwind end. Six behavioural steps were scored: take flight (TF) – insects taking flight and leaving the screen cage; orientation (OR) – moths exhibiting plume searching zigzagging flight and finally locking on to the plume; half-way (HW) – moths flying half-way in the plume between the release point and the stimulus source; source approach (SA) – insects approaching the stimulus source within 10 cm or closer; source contact (SC) – moths touching the stimulus source. Moths were tested 30 min to 4 hours after the beginning of the scotophase. Each individual was tested only once.

Sound stimulus

The sound stimulus consisted of trains of shaped pulses created by multiplication of signals from a pulse generator (HP 8011A) and a sine wave generator (Wavetek 186) in a custom built trapeze modulator. The signal was attenuated (Kay 865 step attenuator), amplified (Xelex power amplifier) and broadcast through a Technics leaf tweeter (EAS10TH400B). The carrying frequency was 20 kHz, which corresponded to the frequency range of best hearing (Skals et al. 2003). In order to calculate the intensity of the sounds, the microphone (with grid on) was calibrated against a G.R.A.S. (Nærum, Danmark) sound calibrator (type 42AB). The frequency response of the microphone is relatively flat (±1 dB) up till 100 kHz. Calibrating the loudspeaker was done several times during the experimental period.

The loudspeaker was positioned along one side of the tunnel at 135 cm distance downwind from the pheromone source and with the middle of the membrane 43 cm above the tunnel floor, thus matching the height of the pheromone plume and the height of the flight path. Sound reflections from the opposite plexiglass wall were minimized covering it with sound absorbing material, which attenuated echoes by at least 20 dB in the middle of the tunnel relative to the situation without this material. The sound absorbing material did not seem to disturb the airflow in the middle of the tunnel as visualised with TiCl4 smoke.

We used pulse trains consisting of 70 pulses, 5 ms long with a repetition rate of 100 pulses/s. The sound pressure level was 87 dB SPL in the middle of the tunnel corresponding to approx. 50 dB above the sensory threshold for the most sensitive cell (Skals et al. 2003). Male and female hearing is identical at 20 kHz where both sexes have a neurophysiological threshold of 38 dB SPL (Skals et al. 2003) and hence we used the same stimulation intensity for both sexes.

In a previous study (Skals et al. in prep.) the sound field inside the tunnel (at normal airspeed) was measured at different heights and distances corresponding approximately to the moth’s position, when it was flying in front of the speaker. At that distance from speaker the intensity 3 cm off axis in the horizontal plane was −5 dB at 20 kHz relative to the on axis intensity. In the vertical plane the intensity 6 cm off axis was −5 dB at 20 kHz. In the direction along the sound propagation axis the sound attenuation followed the inverse square law, i.e. the sound pressure is reduced by 6 dB when the distance is doubled between source and reference point.

Experimental protocol measuring response to sound

When a moth had oriented in the plume and reached approximately half way up to the odour source which corresponded to the position of the loudspeaker the observer turned on the sound stimulus manually simultaneously with a stopwatch. We observed the number of moths that reached the loudspeaker position and the source, and measured the flight duration from the loudspeaker position to the odour source. The procedure was identical in control experiments except that the sound stimulus was not turned on. Moths were allowed 2 min from sound stimulation to reach the rubber septum releasing the odour. Those that did not make it within the time limit contributed 2 min to the
flight duration data. The proportion of animals reaching the odour source was calculated in relation to the number of moths that found the plume and progressed in the plume to the position of the loudspeaker.

Statistical analysis

Number of responses (source contact/no source contact) was compared among sexes and treatments (sound/no sound) using G-test analysis for contingency tables (Fowler et al. 1998). Chi-square analyses for $2 \times 2$ contingency were used to compare control groups with treatment groups (Zar 1984). Differences in flight time from position of loudspeaker to the odour source were compared with ANOVA (Axum ver. 5.0 for windows (MathSoft) followed by Tukeys’ test (Zar 1984). A rejection level of 0.05 was used in all tests.

Results

Dose-response experiment

There was no significant difference between male and female attraction to PAA (chi$^2$-test, df = 1, $\chi^2 = 1.8$, p > 0.05). The proportion of males attracted to 0.1 mg PAA was not different from the proportion attracted to 2.5 $\mu$g pheromone blend (Fig. 1; chi$^2$-test, df = 1, $\chi^2 = 0.55$, p > 0.05). Hence we used these two concentrations in the experiments with sound.

Observations of the behavioural reactions

Moths that were stimulated with sound while flying toward the PAA source reacted to different degrees. Some moths made a small evasive reaction, but did not leave the plume. Others made considerable manoeuvres that would take them out of the plume and back downwind where they would start searching for the odour trail again. Those moths that only showed a small reaction often had difficulties in locating the source at close distance (40 cm) where we often observed casting behaviour and even disrupted flight. Generally, sound stimulation significantly reduced the number of moths that reached the odour source and increased the flight time spend to reach it.

Males flying to PAA

The proportion of males that reached the odour source following sound stimulation was significantly smaller (73%) than in the control group (87%) (chi$^2$-test, df = 1, $\chi^2 = 6.0$, 0.01 < p < 0.05; Fig. 2). Those male moths that reached the rubber septum containing the PAA after sound presentation took significantly longer time to reach the odour source than moths which did not receive any sound (ANOVA followed by Tukeys’ test, p < 0.05; Fig. 3).

Fig. 1. Behavioural response of the silver Y moths to the floral odour phenylacetaldehyde (PAA) and a pheromone blend. Six behavioural steps were scored: take flight (TF), orientation (OR), half-way (HW), source approach (SA) and source contact (SC). The bars represent lumped responses of males and females to different doses of a floral odour. The lines represent the response of males to a two-component pheromone blend. Number of moths in each group is between 30 and 50.

Fig. 2. Proportion of moths that reached the odour source (sex pheromone or floral odour (PAA)) after sound exposure halfway in the tunnel. Filled bars represent the control moths that flew without sound stimulation. Hatched bar represents mated females without sound stimulation. Due to the low n-values these data are not used for statistical evaluation. Values within the bars represent the number of moths in each group. See text for statistical comparisons.
Females flying to PAA
A significantly lower proportion of females reached the PAA source after exposure to ultrasound (74%) compared with females that did not receive sound stimulation (92%; $\chi^2$-test, df = 1, $\chi^2 = 26.3$, $p < 0.001$; Fig. 2). The average flight time to reach the source after sound stimulation was significantly longer than for females in the control group (ANOVA followed by Tukey’s test, $p < 0.05$) because many females made pronounced evasive reactions that made them leave the odour plume (Fig. 3).

Males flying to pheromone
The proportion of males that reached the odour source was significantly reduced from 92% in the control group to 75% in the group that was exposed to sound stimulation ($\chi^2$-test, df = 1, $\chi^2 = 25.6$, $p < 0.001$; Fig. 2). The difference in flight duration was significant between the two groups of moths (ANOVA followed by Tukey’s test, $p < 0.05$; Fig. 3).

Mated females versus non-mated females flying to PAA
Due to rearing problems at the end of this study we did not manage to get sufficient mated females flying to PAA to apply statistical analysis ($n = 6$). Hence we made comparison between mated and virgin females exposed to sound in a $2 \times 2$ contingency table thus ignoring the control groups. The proportion of mated females that reached the odour source after sound stimulation was significantly higher than the proportion of virgin females ($\chi^2$-test, df = 1, $\chi^2 = 15.0$, $p < 0.001$; Fig. 2). Mated females reached the odour source faster when stimulated by sound compared to virgin females, but not significantly faster (ANOVA, followed by Tukey’s test, $p > 0.05$; Fig. 3).

Males versus females
There was no significant difference between the proportion of males and females that reached the source following sound stimulation ($G$-test, df = 3, $G = 0.8$, $p > 0.05$). Likewise, was the duration of flight to the odour source not significantly different between sexes (ANOVA followed by Tukey’s test, $p > 0.05$).

Males attracted to plant odour versus males attracted to pheromone
The proportion of males that reached the plant odour source following sound stimulation did not differ significantly from the proportion of males attracted to the pheromone bait ($G$-test, df = 3, $G = 2.2$, $p > 0.05$). Flight duration following sound stimulation was also independent of whether the odour was a plant odour or sex pheromone (ANOVA followed by Tukey’s test, $p > 0.05$).

Discussion
In this study we examined the effect of ultrasound induced predator defence on olfactory-based foraging and mate-finding behaviour in a prey. Moths stimulated by ultrasound showed a huge variety of behavioural responses as have been observed in field studies (Roeder 1962). When exposed to bat-like sound both male and non-mated female moths reduced their foraging behaviour and males reduced their mate-seeking behaviour. Male and female A. gamma moths respond equally well to acoustic cues mimicking bat predator. Sexual selection theory predicts that males would take higher predation risk than females, which is supported by several studies (Abrahams and Dill 1989, Acharya 1995, Ball and Baker 1996). Other studies have found that females respond more distinctly to predators (Huang and Sih 1990, Magurran and Seghers 1994, Williams et al. 2001). However, the sex-biased predation risk reported in these studies may be due to differences in activity level or sexual selection, since males are competing to locate the calling female. Females are less active than males, and this passive defence may be interpreted as less risk-taking. Hence what may appear, as sex-biased risk taking seems to be more dependent on the behavioural context than on
gender. Our study on *A. gamma* moths suggests that there is no difference in risk-taking when predator defence is examined in identical behavioural context, i.e. foraging flight. However, we cannot exclude that females are taking a just as high risk as males because they might have a stronger need of nectar than males, since they use energy for pheromone calling behaviour and oviposition related behaviour. It has also been shown that if females benefit more than males from foraging then they might take a higher risk of predation (Abrahams and Dill 1989).

Surprisingly we found that sound stimulation had a lower effect on mated females than on non-mated indicating that mated females are more prone to taking higher risks. The percentage of mated females, which reached the odour source after sound stimulation, was almost equal to the percentage of non-mated females, which reached the odour source without sound stimulation. This indicates that mated females are the most active (and risk-taking) category of individuals in the field, at least when they in fact have taken flight towards an odour source. However, mated females might be less active otherwise by e.g. reduced flight time, since males are more prone to being predated (Acharya 1995). When a moth is exposed simultaneously to olfactory and acoustic cues the decision to elicit an escape response is most likely based on a trade-off between the relative intensity of the input to CNS from the two sensory modalities. The adaptive value of flower odour may be higher for mated females, which need to find nectar as fuel for subsequent flight to locate oviposition sites. Risk-taking should be correlated with expected benefits (Magnhagen 1990). Therefore, if females gain higher benefits by foraging on flowers, they might be expected to take a higher risk. Our results suggest, that when females are mated the trade-off mechanism changes in the direction of favouring flower odours relative to acoustic predator cues. It is known that mating may induce some endogenous changes in insects. For example in some species of Lepidoptera mating can enhance oviposition related behaviours (Barton-Browne 1993). However, it is not known if such changes can induce increased sensitivity to floral odours or decreased sensitivity to predatory sounds.

The sound stimuli used in this study were not constructed to imitate a particular bat species. Echolocation signals used by bats for orientation and prey detection vary among species (Simmons et al. 1979, Neuweiler 1990, Fenton 1995). The signals used in this study were constructed to simulate a “high predation risk” signal by mimicking a hunting signal (100 pulses/s) emitted by a “general” sympatric bat when it closes in on airborne targets. As long as the frequency is within the hearing range of the moth, the exact frequency is not important since moths are tone deaf. We used 20 kHz, since this is within the optimal range of hearing in *A. gamma* (Skals et al. 2003) and since it is utilized by sympatric bats, e.g. *Eptesicus serotinus* (Jensen and Miller 1999). The pulse duration used in this study is also within the range utilized by *E. serotinus* (Jensen and Miller 1999). The sound pressure of acoustic stimuli was 87 dB SPL, thus corresponding to a sound level of 50 dB above the neurophysiological threshold level (Skals et al. 2003). Most of the moths in our study reached the odour source even with sound stimulation. However, with a higher sound pressure for sound stimulation we would expect that a smaller proportion of insects would have reached the source. On the other hand, we expected a higher proportion of insects to reach the odour source if the bait contained a higher concentration of the odour. For comparison between the foraging and sexual behaviour we chose odour doses that resulted in similar attraction rate (Fig. 1). In nature, the acoustic and chemical stimuli may vary in intensity with distance to the source and therefore also the decision of whether to respond to the olfactory or the acoustic cue. The optimal solution for moths in these situations is the one that balances costs and benefits to maximize fitness.

The time spent to locate the odour source increased significantly following sound stimulation. The prolonged flight time is caused by the evasive reactions, which often took the moth out of the plume leading it downwind to perform a new search. These findings are in accordance with results reported by Acharya and McNeill (1998) for two different moth species stimulated with simulated bat cries when flying in pheromone plumes. Bat defence behaviour such as evasive manoeuvres taking the moth out of the plume or remaining still and thus cryptic carries the cost of reduced foraging opportunities. Snyder and Wise (2000) showed that beetles reduced the costs of avoiding predators by using the anti-predator behaviours only when predation risk exceeds potential gains from foraging. We suggest that moths make a trade-off between advantages and disadvantages of escaping the bat (surviving) and hence loosing the plume (loss of feeding or mating opportunities) or staying in the plume at increased risk of predation.

From sexual selection theory males are generally expected to take higher risk than females. This is supported by studies that have compared risk-taking in males and females by analysing their defensive responses in different behavioural contexts, which may lead to the false interpretation that sex-biased predator defence is inherited. In this study we have shown that if male and female moths are observed in identical behavioural context then there is no difference in risk-taking. Hence, other traits such as activity level may explain for observed sex-bias in risk-taking in different animals. Therefore, we suggest that risk-taking should be analysed in identical behavioural context when comparing between sexes, ages, species etc.
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